

Primer sequences:

Forward: CACTGCAGCATGATGAAAATAAACG

Reverse: GAATAAAGCTGATAGAGAGTATATG

PCR and restriction digest conditions: The 15 µl PCR reaction contained 50 ng of genomic bovine DNA, 0.2 µM of each primer, deoxynucleotide triphosphates (200 µM), Tris-HCl pH 8.8 (45 mM), (NH₄)₂SO₄ (11 mM), MgCl₂ (4.5 mM), β-2 mercaptoethanol (6.7 mM), ethylenediaminetetraacetic acid (4.5 mM), spermidine (0.25 mM) and 0.65 U *Taq* DNA polymerase (Invitrogen, Burlington, ON, Canada). The cycling protocol was 3 min at 94 °C, 30 cycles of 94 °C for 45 s, 52 °C for 45 s, 72 °C for 30 s, with a final extension at 72 °C for 3 min. A 2-h digestion with *Mly*I (New England Biolabs, Mississauga, ON, Canada) was carried out in a 37 °C waterbath. The digested PCR products were separated on a 2% agarose gel.

Polymorphisms: Three single nucleotide polymorphisms were detected within intron 2. At nucleotide (nt) 284 (GenBank accession no. AY491054), a cytosine was substituted with an adenine; at nt 666, a thymine was substituted with an adenine; at nt 3032, a cytosine was substituted with a thymine. The third SNP introduced a recognition site for *Mly*I. When amplified with the reported set of primers the SNP was at base pair (bp) 193 of the PCR product. The PCR product of 289 bp was restricted into two fragments of 199 and 90 bp when thymine was present at the SNP site (Fig. 1).

Mendelian inheritance: Segregation was consistent with co-dominant inheritance and was found in 19 families screened.

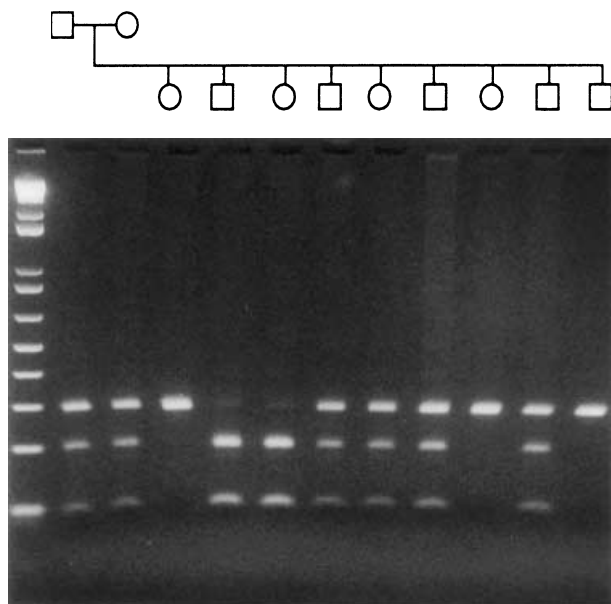


Figure 1 A polymerase chain reaction-restriction fragment length polymorphism in the bovine *neuropeptide Y* gene segregates through an embryo-transfer family from a Charolais dam and a Limousin sire. Left lane = 1-kb plus ladder (Invitrogen). The uncut allele is 289 bp and the cut allele includes fragments at 199 and 90 bp.

Chromosomal location: The Canadian Beef Cattle Reference Herd³ was used for linkage mapping. As part of a larger quantitative trait loci study, 162 microsatellites, six of which were on cattle chromosome (BTA) 4, were used to genotype 20 parents and 136 offspring. NPY was mapped 8 cM from *RM067* (LOD = 10.19) and 1 cM from *BM6458* (LOD = 21.02) using the 'TWO POINT' function of CRI-MAP (version 2.4; St Louis, MO, USA). The most likely order using the 'ALL' function was *BL1024-RM188-RM067-NPY-BM6458-BMS648-BR6303*. When including the data generated from the mapping of *IGFBP3* to cattle chromosome 4,⁴ the most likely order using the 'ALL' function was *BL1024-RM188-RM067-NPY-BM6458-IGFBP3-BMS648-BR6303*. This assignment is in agreement with the localization of NPY to human chromosome 7p15,⁵ mouse chromosome 6⁶ and rat chromosome 4.⁷

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Male-specific *SRY* and *ZFY* haplotypes in US beef cattle

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Source/description: The zinc finger Y-linked gene (*ZFY*) is thought to code for a transcriptional activator¹ and has been mapped to a distal region of the bovine Y chromosome short arm (Yp13).² *ZFY* has an X-linked homologue, *ZFX*, from which *ZFY* originates.³ A male-specific primer pair has been previously reported⁴ that amplifies a 979 bp portion of the bovine *ZFY* gene in a DNA panel of 96 bulls repre-

senting 17 breeds of cattle [Meat Animal Research Center (MARC) Beef Cattle Diversity Panel 2.1 (MBCDP2.1)⁵]. This primer pair, multiplexed with ZFX primers, comprises a PCR assay that detects portions of the bovine X- and Y-chromosomes.⁴

The sex-determining region of the Y-chromosome (SRY) codes for a transcription factor that is critical for testis differentiation.⁶ The bovine SRY gene has been mapped to two putative locations by radiation hybrid mapping, one lies in the short arm of the Y-chromosome (Yp) near the pseudo-autosomal boundary region and the other in the distal region of the long arm (Yq).⁷ Both locations are contained within the male-specific region of the Y-chromosome; thus, SRY is typically found only in males. Here, we describe the male-specific amplification and sequence analysis of a 1348 bp portion of SRY in all 96 DNA samples comprising MBCDP2.1, and Y-chromosome haplotypes involving SRY and ZFY alleles in US beef cattle.

Primers:

SRY-sense 26404: 5'-ACA GAG ACT ACT AGC CAT ACA C-3'
SRY-antisense 26414: 5'-CAA TTT TTC TAC TTT AGC CTA T-3'

ZFY-sense 8761: 5'-GGT GAG GGC ACA TGA GTT C-3'

ZFY-antisense 8764: 5'-CTC TGC AGG TGG TTG TGT AA-3'

PCR conditions and sequencing: ZFY PCR was performed as described⁴ in total volumes of 50 µl containing 50 ng genomic DNA, 1.25 units of either HotStarTaq DNA polymerase (Qiagen, Inc., Valencia, CA, USA) or Thermo-Start DNA polymerase (ABgene, Epsom, UK), 1.5 mM MgCl₂, 200 µM dNTPs, 10% v/v reaction buffer provided by the manufacturer,

0.5 µM of sense, and 0.2 µM of antisense amplification primers. SRY PCR was performed in total volumes of 55 µl containing 50 ng genomic DNA, 1.25 units of Thermo-Start DNA polymerase, 2.4 mM MgCl₂, 184 µM dNTPs, 10% v/v reaction buffer and 0.4 µM of amplification primers.

Thermocycling conditions for both SRY and ZFY PCRs were as follows: 94 °C for 15 min, 45 cycles of 94 °C for 20 s, 58 °C for 30 s, 72 °C for 1 min, and a final incubation at 72 °C for 3 min. Amplifications were performed in either a PTC-200, PTC-220, or PTC-225 dyad thermocycler (MJ Research, Watertown, MA, USA). Five microlitres of each sample was analysed for amplification products post-PCR by agarose gel electrophoresis. Amplicons were purified with Qiagen DNA purification columns (Qiagen) and sequenced with amplification or nested sequencing primers, BigDye terminator chemistry and an ABI 3700 capillary sequencer (PE Applied Biosystems, Boston, MA, USA).

Specificity of SRY and ZFY primers for the Y-chromosome: Genomic DNA from 47 Hereford males and 41 Hereford females of the MARC reference population⁸ was tested for PCR amplification with the SRY primer pair. Genomic DNA from the entire MARC reference population (149 males, 265 females) was tested for PCR amplification with the ZFY primer pair. The male DNA samples yielded correctly sized SRY or ZFY amplicons as determined by agarose gel electrophoresis. In contrast, SRY and ZFY amplicons were not detected in samples containing female DNA (data not shown).

Sequence analysis: Sequences from the 96 bulls of MBCDP2.1 were aligned and analysed with Phred and Phrap,^{9,10} Polyphred 3.0, and Consed 8.0¹¹ software. Analysis of the 979 bp

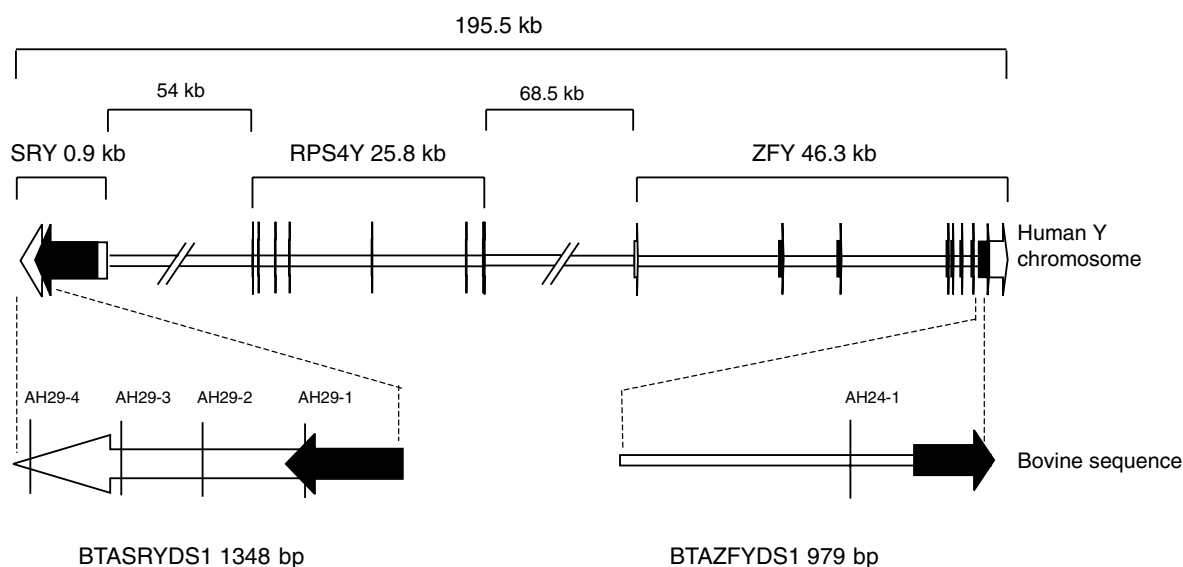


Figure 1 Physical maps of human Y-chromosome partial DNA sequence (GenBank accession no. NT_011896) and two PCR amplicons (BTASRYDS1 and BTAZFYDS1). Coding sequence, untranslated regions, and introns are represented by black arrows, white arrows, and thin rectangles respectively. The diagonal lines on the human intergenic region indicate places in the physical map that are not to scale. Dashed lines indicate homology of the human Y-chromosome with bovine DNA amplification products. Numbered vertical lines on BTASRYDS1 and BTAZFYDS1 indicate the position of SNPs.

Table 1 Allele frequencies and haplotypes¹ of bovine SRY and ZFY SNPs in the multibreed panel MBCDP2.1.

Animal group ²	Number of animals	SRY SNPs						ZFY SNP ³			
		AH29-1		AH29-2		AH29-3		AH29-4		AH24-1	
		TCCCT[G,T]TAACA ⁴		GGTAG[C,T]ATATA		TATAG[T,C]CTTTT		GATAA[A,G]CAAGA		GTCAT[C,T]ATGAG	
		G	T	C	T	T	C	A	G	C	T
Angus	8	1.00	— ⁵	1.00	—	1.00	—	1.00	—	1.00	—
Hereford	8	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—
Limousin	8	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—
Simmental	7	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—
Charolais	6	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—
Beefmaster	5	0.60	0.40	0.60	0.40	0.60	0.40	0.60	0.40	0.60	0.40
Red Angus	6	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—
Gelbvieh	6	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—
Brangus	5	0.80	0.20	0.80	0.20	0.80	0.20	0.80	0.20	0.80	0.20
Salers	5	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—
Brahman	6	—	1.00	—	1.00	—	1.00	—	1.00	—	1.00
Shorthorn	5	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—
Maine-Anjou	5	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—
Longhorn	4	0.25	0.75	0.25	0.75	0.25	0.75	0.25	0.75	0.25	0.75
St Gertrudis	4	—	1.00	—	1.00	—	1.00	—	1.00	—	1.00
Chianina	4	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—
Holstein	4	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—
Total	96	0.83	0.17	0.83	0.17	0.83	0.17	0.83	0.17	0.83	0.17

¹Haplotypes are concatenated SNPs AH29-1 through AH29-4, and AH24-1. Haplotype sequences are the following: 1-GCTAC, 2-TTCGT.

²Animals are from the multibreed panel MBCDP2.1.

³Previously published,⁴ GenBank accession no. AF465181.

⁴Five nucleotides flanking each side of the polymorphic site (e.g.[G,T]) are presented.

⁵A frequency of zero is denoted with a dash.

amplicon of ZFY from all 96 bulls (GenBank accession no. AF465181) identified two haplotypes defined by one single nucleotide polymorphism (SNP) within intronic sequence (AH24-1)⁴ (Fig. 1). One haplotype is prevalent in the US Brahman breed and Brahman influenced composites (a limited representation of the *Bos indicus* lineage), the other haplotype is prevalent in *B. taurus* breeds.

Four SRY SNPs were identified from individuals of the multibreed panel (Fig. 1). One SNP (AH29-1) resides in the second position of codon 214 (C214F). The remaining three SNPs are located in the 3'-UTR. Two sets of four SRY SNP alleles in linkage disequilibrium (LD) that are additionally in LD with a ZFY allele were observed in the animals comprising MBCDP2.1. Consequently, two male-specific haplotypes of the US beef cattle are defined by the SRY and ZFY regions we examined (Table 1). Nucleotides at four monomorphic sites of our SRY consensus sequence (representing the 96 bulls of MBCDP2.1, GenBank accession no. AY189027) were different from corresponding nucleotides in two bovine SRY sequences in GenBank (AB039748, E11537). The reason for these differences is not known; however, the total variation within the SRY gene is not expected to be captured by our panel of US beef cattle.

Comments: The PCRs described in this study are Y chromosome-specific and amplify a portion of the SRY and ZFY genes in 16 beef breeds and one dairy breed. Either of these haplotype assays can be used to determine the sex of cattle DNA from diverse US beef herds. Six theoretical haplotypes are predicted from five bi-allelic SNPs assuming sequential allele mutation and barring recombination.¹² The LD of SRY and ZFY alleles observed in this study indicates that four predicted Y haplotypes are not present in more than 98% of US beef cattle overall. Analysis of additional male-specific SNPs is needed to resolve the patterns of paternal lineage in US beef cattle.

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Names are necessary to accurately report the available results; however, the USDA neither guarantees nor warrants the standard of the product, and the use of names by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Assignment of 10 canine genes to the canine linkage and comparative maps

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Source/description: Linkage maps were made for five canine chromosomes (3, 4, 11, 25 and 26), containing 10 new gene markers among 39 published markers (Fig. 1). Canine genomic sequences for *HAND2* (GenBank accession no. AACN010789975), *MEF2C* (AACN010136027 and AACN010131210), *TBX5* (AACN010089531), *NKX2-5* (AACN010634695 and CE799216), *NCOR2* (AACN010005092), *TGFBI* (AACN010065942), *HTR4* (AACN010660515) and *RAI14* (AACN010129610), were obtained by searching the 1.5× canine genomic sequence¹ with the corresponding human gene sequences. The dog sequences were then verified by BLAST searches (<http://www.ncbi.nlm.nih.gov/blast/>) to be from the orthologous gene. Primers were designed from these sequences that allowed the amplification of simple sequence repeats or gene introns. The PCR products were sequenced from two founder dogs to identify polymorphisms. Six of the eight polymorphisms identified were based on simple sequence repeats, and two on single nucleotide polymorphisms (SNPs). A simple sequence repeat near the *ARSB* locus was identified in a cosmid clone isolated using a canine *ARSB* PCR amplified cDNA probe (P. Foureman personal